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6. AUTHORS
Steven J Schwartz

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Ohio State University Research Foundation
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14. ABSTRACT
This project involves an Industry/University Cooperative Research Center consortium between The Ohio State University, the University of California, Davis, and North Carolina State University to assist in advancing food processing and packaging technology and will greatly benefit the US food industry and military personnel. This industry driven consortia center, CAPPS – Center for Advanced Processing and Packaging Studies, focuses on industrial relevant research directed toward coupling microbial life sciences with process and package engineering

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Report Title

Final Report for the Center for Advanced Processing and Packaging Studies (CAPPS)

ABSTRACT

This project involves an Industry/University Cooperative Research Center consortium between The Ohio State University, the University of California, Davis, and North Carolina State University to assist in advancing food processing and packaging technology and will greatly benefit the US food industry and military personnel. This industry driven consortia center, CAPPS – Center for Advanced Processing and Packaging Studies, focuses on industrial relevant research directed toward coupling microbial life sciences with process and package engineering to assure food safety. The objectives of CAPPS are to enhance safety and quality of aseptic and extended shelf-life products, to characterize emerging, aseptic and extended shelf-life processes, and to assure the integrity and functionality of aseptic and extended shelf-life packaging. Examples of new strategic initiatives for the center include development of ingredient and dry powder sterilization technologies, new retort methods, pre-treatment for novel processes, in-package ohmic sterilization, RFID technologies, and research on effective nutrient delivery. The involvement of governmental agencies such as US Army Natick within the Center enhances the capabilities of the program to include critical needs of military personnel and assist in directing new technologies with dual use.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

1.Wannasawat Ratphitagsanti, Silvia De Lamo-Castellvi, V.M. Balasubramaniam, and Ahmed Elmeleigy Yousef. 2010. Efficacy of pressure-assisted thermal processing, in combination with organic acids, against Bacillus amyloliquefaciens spores suspended in deionized water and carrot puree. Journal of Food Science. Vol. 75, no. 1: M46-M52.

2.Ratphitagsanti, W., Ahn, J., V.M. Balasubramaniam, and A.E. Yousef. 2009. Influence of pressurization rate and pressure pulsing on the inactivation of bacterial spores during pressure-assisted thermal processing. Journal of Food Protection.Vol. 72, no. 4: 775-782.

3.Balasubramaniam, V.M. and D. Farkas. 2008. High pressure food processing. Food Science and Technology International. Vol. 14, no. 5: 413-418.

4.Balasubramaniam, V.M, Farkas, D., and Turek, E. J. 2008. Preserving foods through high-pressure processing. Food Technology. Vol. 62, no. 11. (November): 32-38.

5.Ahn, J.; V.M. Balasubramaniam; and A.E. Yousef. 2007. Inactivation kinetics of selected aerobic and anaerobic bacterial spores by pressure-assisted thermal processing. International Journal of Food Microbiology. Vol. 113, no. 3: 321-329.

6.Ahn, Juhee; and Balasubramaniam, V. M. 2007. Physiological responses of Bacillus amyloliquefaciens spores to high pressure. Journal of Microbiology and Biotechnology. Vol. 17, no. 3: 524-529.

7.Ahn, Juhee; and V.M. Balasubramaniam. 2007. Effects of inoculum level and pressure pulse on the inactivation of Clostridium sporogenes spores by pressure-assisted thermal processing. Journal of Microbiology and Biotechnology. Vol. 17, no. 4: 616-623.

8.Barrett D, Beaulieu J, Shewfelt R. Color, Flavor, Texture, and Nutritional Quality of Fresh-Cut Fruits and Vegetables: Desirable Levels, Instrumental and Sensory Measurement, and the Effects of Processing. Critical Revs. in Food Sc. & Nutrition. 2010 5;50(5):369-389.

9.Asavasanti S, Ersus S, Ristenpart W, Stroeve P, Barrett DM. Critical Electric Field Strengths of Onion Tissues Treated by Pulsed Electric Fields. Journal of Food Science. 2010 9;75(7):E433-E443.

10.Ersus S, Oztop MH, McCarthy MJ, Barrett DM. Disintegration Efficiency of Pulsed Electric Field Induced Effects on Onion (Allium cepa L.) Tissues as a Function of Pulse Protocol and Determination of Cell Integrity by 1H-NMR Relaxometry. Journal of Food Science. 2010 9;75(7):E444-E452.

11.Gonzalez ME, Barrett DM. Thermal, High Pressure, and Electric Field Processing Effects on Plant Cell Membrane Integrity and Relevance to Fruit and Vegetable Quality. Journal of Food Science. 2010 9;75(7):R121-R130.

12.Gonzalez ME, Anthon GE, Barrett DM. Onion Cells After High Pressure and Thermal Processing: Comparison of Membrane Integrity Changes Using Different Analytical Methods and Impact on Tissue Texture. Journal of Food Science. 2010 9;75(7):E426-E432.

13.Gonzalez M, Jernstedt J, Slaughter D, Barrett D. Microscopic Quantification of Cell Integrity in Raw and Processed Onion Parenchyma Cells. Journal of Food Science. 2010 9;75(7):E402-E408.

14.Gonzalez M, Jernstedt J, Slaughter D, Barrett D. Influence of Cell Integrity on Textural Properties of Raw, High Pressure, and Thermally Processed Onions. Journal of Food Science. 2010 9;75(7):E409-E416.

15.Gonzalez ME, Barrett DM, McCarthy MJ, Vergeldt FJ, Gerkema E, Matser AM, et al. 1H-NMR Study of the Impact of High Pressure and Thermal Processing on Cell Membrane Integrity of Onions. Journal of Food Science. 2010 9;75(7):E417-E425.

Number of Papers published in peer-reviewed journals: 15.00

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Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Daryaei, H., Balasubramaniam, V.M., and AE Yousef. 2010. Lethality enhancement of pressure-assisted thermal food processing against Bacillus amyloliquefaciens spores using antimicrobial compounds [Abstract]. Abstract no. 042-01. 2010 Annual Meeting of Institute of Food Technologists. July 17-20. Chicago, IL [Peer Reviewed] (Published)

Loc Thai Nguyen, V.M. Balasubramaniam, and Wannasawat Ratphitagsanti. 2010. Modeling accumulated lethality during pressure-assisted thermal processing [Abstract]. Abstract no. 071-19. 2010 Annual Meeting of Institute of Food Technologists. July 17-20. Chicago, IL [Peer Reviewed] (Published)

Ratphitagsanti Wannasawat, Luis E. Rodriguez-Saona, and V. M. Balasubramaniam. 2009. Characterization of Bacillus amyloliquefaciens spores after thermal and pressure-assisted thermal processing by infrared microspectroscopy and multivariate analysis [Abstract]. Abstract 125-04. 2009 Annual Meeting, Institute of Food Technologists. June 5-10. Anaheim, CA [Peer Reviewed] (Published)

Ratphitagsanti Wannasawat, Luis E. Rodriguez-Saona and V.M. Balasubramaniam. 2009. Impact of Pressure Pulsing on Biochemical Changes of Bacillus amyloliquefaciens Spore Inactivation through Fourier Transform Infrared Microspectroscopy [Abstract]. Abstract P3-92. 2009 Annual Meeting, International Association for Food Protection. July 12-15. Grape Vine, TX [Peer Reviewed] (Published)

Lamo-Castellviastellvi,S. Ratphtagsanti,,W. Balasubramaniam,V.M., and Yousef, A. E. 2008. Combined effects of sucrose laurate ester and pressure-assisted thermal processing to inactivate Bacillus amyloliquefaciens spores suspended in mashed carrots. [Abstract]. No. T4-07. 95th Annual Meeting of International Association of Food Protection,. August 3-6. Columbus,, Ohio [Peer Reviewed] (Published)

Ratphitagsanti Wannasawat, Silvia Delamo-Castellvi, V.M. Balasubramaniam, and Ahmed Elmeleigy Yousef. 2008. Effect of organic acids on Bacillus amyloliquefaciens inactivation by pressure-assisted thermal processing [Abstract]. Abstract no. 171-04 2008 Annual Meeting of Institute of Food Technologists. June 28- July 1. New Orleans,, LA [Peer Reviewed] (Published)

E. Park, W. Ratphitagsanti, P. Chiang, C.S. Lee, J. Lee. Rapid Detection of Spore Contamination in Water, Beverage, and Powder by ATP luminescence and Real-time PCR. Institute of Food Technologists Annual Meeting. Chicago, Illinois, July 17-20, 2010.

Kumar, P., Reinitz, H.W., Simunovic, J., Sandeep, K.P., Franzon, P.D. 2009. Overview of RFID technology and its applications in the food industry. Journal of Food Science: Concise reviews and hypotheses in Food Science. Vol. 74(8): R101-R106.

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 8

(d) Manuscripts

Ersus, S. & Barrett, D.M., Determination of membrane integrity in onion tissues treated by pulsed electric fields: Use of microscopic images and ion leakage measurements, Innovative Food Science and Emerging Technologies (2010)

W. Ratphitagsanti, E. Park, C.S. Lee, R-Y. A. Wu, J. Lee. High-throughput detection of spore contamination in food packages and food powers using tiered approach of ATP bioluminescence and real-time PCR. (submitted in 2010)

Number of Manuscripts: 2.00

Patents Submitted

Balasubramaniam, V.M., Sastry, S.K. and Sunghee Park., Primary inventor. filed 2009. (patent pending) Pressure-ohmic thermal sterilization. patent no. invention disclosure submitted with university, patent application is being processed.

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME PERCENT_SUPPORTED

FTE Equivalent:

Total Number:

Sub Contractors (DD882)

Inventions (DD882)

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List of papers submitted or published resulting from CAPPS support from 2007-2010:

Peer Reviewed Papers:

1. Wannasawat Ratphitagsanti, Silvia De Lamo-Castellvi, V.M. Balasubramaniam, and Ahmed Elmeleigy Yousef. 2010. Efficacy of pressure-assisted thermal processing, in combination with organic acids, against Bacillus amyloliquefaciens spores suspended in deionized water and carrot puree. Journal of Food Science. Vol. 75, no. 1: M46-M52.

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Accepted manuscript:

Ersus, S. & Barrett, D.M., Determination of membrane integrity in onion tissues treated by pulsed electric fields: Use of microscopic images and ion leakage measurements, Innovative Food Science and Emerging Technologies (2010)

Peer reviewed papers under review:

W. Ratphitagsanti, E. Park, C.S. Lee, R-Y. A. Wu, J. Lee. High-throughput detection of spore contamination in food packages and food powers using tiered approach of ATP bioluminescence and real-time PCR. (submitted in 2010)

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2. Gupta Rockendra, Galina Mikhaylenko, V.M. Balasubramaniam, and Juming Tang. 2010. Combined pressure-temperature effects on the kinetics of chemical marker (4-hydroxy,5-methyl, 3(2H)-furanone) formation in whey protein gels [Abstract].Abstract no. 236-43. 2010 Annual Meeting of Institute of Food Technologists. July 17-20. Chicago, IL [Peer Reviewed] (Published)

3. Loc Thai Nguyen, V.M. Balasubramaniam, and Wannasawat Ratphitagsanti. 2010. Modeling accumulated lethality during pressure-assisted thermal processing [Abstract]. Abstract no. 071-19. 2010 Annual Meeting of Institute of Food Technologists. July 17-20. Chicago, IL [Peer Reviewed] (Published)

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7. Ratphitagsanti Wannasawat, Silvia Delamo-Castellvi, V.M. Balasubramaniam, and Ahmed Elmeleigy Yousef. 2008. Effect of organic acids on Bacillus amyloliquefaciens inactivation by pressure-assisted thermal processing [Abstract]. Abstract no. 171-04 2008 Annual Meeting of Institute of Food Technologists. June 28- July 1. New Orleans,, LA [Peer Reviewed] (Published)

8. E. Park, W. Ratphitagsanti, P. Chiang, C.S. Lee, J. Lee. Rapid Detection of Spore Contamination in Water, Beverage, and Powder by ATP luminescence and Real-time PCR. Institute of Food Technologists Annual Meeting. Chicago, Illinois, July 17-20, 2010.

9. Kumar, P., Reinitz, H.W., Simunovic, J., Sandeep, K.P., Franzon, P.D. 2009. Overview of RFID technology and its applications in the food industry. Journal of Food Science: Concise reviews and hypotheses in Food Science. Vol. 74(8): R101-R106.

Books:

Zhang, H. Q., G. Barbosa-Canovas, V.M. Balasubramaniam, P. Dunne, D. Farkas, and J. Yuan. 2010. Handbook of Nonthermal Processing Technologies for Food. Chicago, il: Blackwell Publishing. (In press)

Book Chapters:

1. Nguyen, L. T., and V.M. Balasubramaniam. 2010. Fundamentals aspects of food preservation with high-pressure processing. In Handbook of Nonthermal Processing Technologies for Food. Edited by Zhang, H. Q., Barbosa-Canovas, G. V., Balasubramaniam, V., Dunne, P., D. Farkas, and Yuan, J. Chicago, il: Blackwell. (In press)

2. V.M. Balasubramaniam. 2010. High Pressure Food Preservation. In Encyclopedia of Agricultural, Food, and Biological Engineering. Edited by Dennis R. Heldman and Carmen Moraru. New York, NY: Taylor & Francis. (In press)

3. Wannasawat Ratphitagsanti, Silvia De Lamo-Castellviand and V.M. Balasubramaniam. 2008. Biological spore inactivation by pressure-assisted thermal processing: Challenges in finding a suitable biological indicator for process validation. In Biological indicators for sterilization processes. Edited by Margarita Gomez and Jeanne Moldenhauer. River Grove, IL: Parenteral Drug Association and Davis Healthcare International Publishing. 413-450.

Inventions:

Balasubramaniam, V.M., Sastry, S.K. and Sunghee Park., Primary inventor. filed 2009. (patent pending) Pressure-ohmic thermal sterilization. patent no. invention disclosure submitted with university, patent application is being processed.

Graduate Students Supported:

Ratphitagsanti Wannasawat, OSU
Rockendra Gupta, OSU
Loc Nguyen, OSU
Akalu Lentiro, NCSU

Post Doctorates Supported:
Silvia Delamo-Castellvi, OSU
Juhee Ahn, OSU
Hossein Daryaei, OSU

Undergraduates Supported:
Chelsea Johnson, OSU

Other Research Staff Supported:
Harry Reinitz, NCSU

Number of Peer Reviewed Papers: Fifteen
Papers published in peer-reviewed journals Fifteen
Number of Presentations: Nine
Number of Manuscripts: One
Manuscripts submitted, but not published (N/A for none) One
Number of Books: One
(d) Books (N/A for none)

Inventions One **Inventors** One

Graduate Students Four
Faculty
Post Doctorates Three
Under Graduates One
Other Research Staff One

The following report is for the Center for Advanced Processing and Packaging Studies (CAPPS). US Army Natick Soldier RD&E is one of ten industrial and government members of this center. Membership fees in the CAPPS consortium are pooled to provide funds for research. Membership fees are set at $35,000 per year and each member has the option to additionally fund separate enhancement projects within the Center.

High pressure inactivation of noroviruses in aqueous medium and purees

Fangfei Lou[1], Hudaa Neetoo[2], Haiqiang Chen[2] and Jianrong Li[1]

[1]The Ohio State University, Department of Food Science and Technology, Columbus, OH, USA; [2]University of Delaware, Department of Animal and Food Sciences, Newark, DE, USA.

***Introduction*:** Fresh produce and related products are often high-risk food because they can become contaminated at pre-harvest and post-harvest stages and they undergo minimal or no processing. Norovirus is the top cause of produce outbreaks (40%), followed by *Salmonella* (18%), *E. coli* (8%), *Clostridium* (6%) and hepatitis A virus (4%). Noroviruses account for more than 39% of outbreaks in fruits and other related products such as puree and juice. Therefore, there is an urgent need to develop non-thermal processing technologies to inactivate foodborne enteric viruses in vegetables, fruits, and purees.

Purpose: The objective of this study was to determine the effectiveness of high pressure processing (HPP) on inactivation of noroviruses in aqueous medium and purees.

Methods: The cultivable murine norovirus was inoculated into Dulbecco's Modified Eagle Medium (DMEM) and purees to a final concentration of approximately 10^7 Plaque forming Unit (PFU)/ml or 10^7 PFU/g. The samples were treated at pressures ranging from 200-450 MPa at either 4 or 20 ^oC for 2 min. The virus survivors were quantified by viral plaque assay and the inactivation kinetics of norovirus were determined.

Results:

1. Pressure inactivation of norovirus in aqueous medium. We first aimed to determine whether HPP effectively inactivated norovirus in aqueous medium. We found that pressure, pH and temperature significantly affected the inactivation of norovirus in cell culture medium. First of all, viral titer was gradually decreased when the pressure was increased. Second, norovirus was easier to be inactivated by HPP at 4 ^oC than at 20 ^oC. Third, norovirus was more sensitive to HPP at pH 7.0 than at pH 4.0. Under pressure above 350 MPa for 2 min, viruses were almost completely inactivated (less than 1 log survivor) at pH 7.0 and 4 ^oC.

2. Inactivation of norovirus in strawberry puree. We have determined the effectiveness of norovirus inactivation in strawberry puree. Viruses were inoculated into strawberry puree at final concentration of 10^7 PFU/g, and the samples were subjected to three different pressures (300, 350, and 400MPa) at either 4^oC or 20 ^oC for 2 min. In all cases, noroviruses were more sensitive to HPP at 4 ^oC than at 20 ^oC. Approximately 5 log virus reduction was achieved under 450MPa at 4^oC. However, only 4 log virus reduction was observed under the same pressure at 20^oC. Again, these results demonstrated that norovirus can be more effectively killed by HPP at higher pressure (450MPa) and lower temperature (4^oC).

3. The effect of pH on the inactivation of norovirus in strawberry puree. The natural pH of strawberry puree is around 3.5. To further investigate whether pH plays a role in inactivation of virus in the same food matrix, we adjusted the pH of strawberry puree from 2.5 to 6.5. The samples

were pressurized at 400 MPa and 4 ^{o}C for 2 min. We found that norovirus was easier to be inactivated at a higher pH. Approximately 5 log virus reduction was achieved at pH 6.5. However, only 2.8 log virus reduction was observed at pH 2.5. Taken together, these results demonstrate that pH significantly affects the virus inactivation in strawberry puree.

4. The effectiveness of norovirus inactivation by HPP in different purees. Next, we tested norovirus survival in a number of purees at two pressures (350 and 400 MPa) at 4 ^{o}C for 2 min. These purees include lemon (pH 2.5), tomato (pH 4.5), strawberry (pH 3.6), watermelon (pH 5.3), carrot (pH 5.75), and carrots juice (pH 6.3). With the exception of lemon puree, the effectiveness of virus inactivation appeared to be correlated with the natural pH in puree (Fig.1). The higher pH of the puree was, the higher log reduction in virus was. We observed 3-4 log and 5-7 log virus reduction at 350MPa and 400MPa, respectively. However, a 5.5 log virus reduction was observed in lemon puree at 350 MPa even at low pH (2.5), providing evidence that food matrix plays an important role in protecting virus from inactivation. Taken together, these results suggest that both pH and food matrix affect the norovirus inactivation in puree.

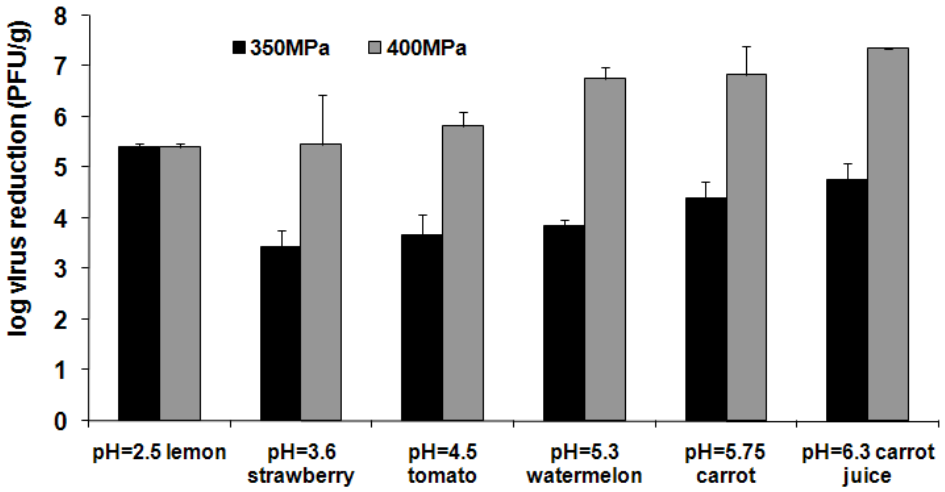


Figure 1 Inactivation of norovirus in different purees

Conclusion*:* HPP effectively inactivates norovirus in aqueous medium and purees. Pressure, temperature, pH, and food matrix affect the effectiveness of virus inactivation by HPP. Under our experimental conditions, HPP did not affect the texture and other sensory qualities such as color and freshness. Thus, HPP is a novel intervention for processing fruits intended for frozen storage and fruit products such as puree, sauce, and juice.

Future direction: We are currently studying the mechanism of viral inactivation by HPP. We will systemically examine how HPP affects viral receptor binding, viral replication and gene expression. Using human norovirus, we will determine the properties of viral caspid and genetic material after HPP.

Oxidative Stability of Food Bioactives in Micro and Nano Encapsulated Particles

N. Nitin, Food Science and Technology, UC Davis

Progress Report- March, 2010

- *Objectives: The specific objectives of the research are:*

Aim 1: Develop an assay to characterize diffusion of oxygen through a selected encapsulation barrier:

Aim 2: Compare the oxygen diffusion barrier properties of microemulsion and microparticles

Approach: In this seed proposal, the research was focused on development of an assay to characterize diffusion of oxygen through a selected encapsulation barrier. To demonstrate working principle of this assay and to compare microencapsulation barriers, we selected microemulsions as a model system for initial studies. Microemulsions provide flexibility in engineering composition and an established approach to deliver bioactive compounds. In the next section results are presented for WPI microemulsions with and without crosslinking and SDS microemulsions with and without chitosan coatings. WPI microemulsions were used to establish an assay to characterize diffusion of oxygen through a selected encapsulation barrier and the results of WPI microemulsions were compared with SDS microemulsions with and witout chitosan coatings.

Aim 1: Develop an assay to characterize diffusion of oxygen through a selected encapsulation barrier:

The particle size was analyzed using both light scattering measurements and microscopy. Table 1 shows the hydrodynamic particle diameter for various microemulsions measured using a light scattering method. although the mean diameter changes with a given formulation, the overall distribution of particle size is similar across

various emulsions excluding SDS- Chitosan. This has been represented in the table by evaluating % of total population below 50 microns. Figure 1 shows the microscopic images of these emulsions. In this study, chemical cross-linking approach was selected to decrease the mobility of WPI molecules on microemulsion droplets. Chemical cross-linking of WPI microemulsion droplets results in an increase in average particle size as compared to non-crosslinked WPI microemulsions. This increase in hydrodynamic diameter of microemulsion droplets after chemical cross-linking can be explained by an increase in inter-particle association of microemulsion droplets as observed using light microscopy. This increased inter-particle association can be induced by a presence of small amount of excess whey protein in solution that can induce bridging of droplets.

In this study, SDS microemulsion droplets were modified using a layer-by layer assembly of chitosan biopolymer based on electrostatic interaction. This approach was selected to evaluate the role of coatings on microemulsion droplets to limit transport of oxygen across microemulsion barriers. Addition of chitosan to the SDS emulsion shows a significant increase in the particle size based on light scattering measurements. However, the microscopic images show no significant change in microemulsion droplet size as compared with SDS microemulsion droplets. This discrepancy between the light scattering and the light microscopy measurementscan be explained based on flocculation of microemulsion droplets in presence of chitosan. In this scenario, chitosan polymer due to its large molecular weight (~300-500 k Da) and extended chain length can associate with multiple droplets resulting in flocculation which leads to an increase in particle size measured using light scattering. Similar observation has been reported by Helgason et al. (2009) for SDS-chitosan microemulsions.

After particle size characterization of microemulsions, experiments were conducted to quantify the rate of transport of oxygen across microemulsion barriers. Figure 2a shows the decay in fluorescence intensity of oxygen sensitive dye encapsulated in the nitrogen purged WPI microemulsion droplets upon exposure to air. The decrease in fluorescence intensity of nitrogen purged WPI microemulsion was compared with air purged (control) WPI microemulsion. The average maximum fluorescence of nitrogen purged microemulsion at time t=0 s was 2.7 times the fluorescence of the control sample, indicating a good dynamic range for the system.

Similar ratio between a nitrogen purged and air exposed dye sample has been reported by Watkins and others (1998). These results validate that the microemulsions were purged adequately and the residual oxygen concentration in the emulsion was minimum.

The initial decrease in fluorescence intensity of nitrogen purged WPI microemulsion upon exposure to air was followed by a slower decline and an eventual pseudo-steady state level in the fluorescence intensity over an extended period of time (24hr) (figure 2b). Rapid loss of fluorescence in nitrogen purged samples upon exposure to air indicates transport of oxygen molecules from air to microemulsion droplets across WPI barrier. The control sample showed a relatively constant fluorescence value during this measurement. The slight decrease in the fluorescence values for control can be attributed to photo-bleaching of the dye as a result of exposure to the light from the instrument during measurements. Since the solubility of oxygen in food grade oils is approximately three times as compared to aqueous solutions (Coupland and McClements, 1996), it is expected that in the absence of significant barrier across microemulsions the fluorescence will continue to decrease and reach equilibrium levels of fluorescence intensity i.e. similar to control sample saturated with air. Additionally, due to increased surface area of oil droplets in microemulsions, oxygen transport rates from surrounding environment to oil droplets are expected to increase as compared to bulk oils. Microemulsions with good barrier properties to oxygen transport are expected to decrease the rate of decline in fluorescence intensity. Rapid decrease followed by a slow decline in fluorescence intensity indicates that WPI coating of microemulsion droplets provides a partial barrier to oxygen transport. In initial stages, large concentration gradient in oxygen results in rapid transport of oxygen followed by a slow and a restricted transport across microemulsion barrier with a decreasing gradient in oxygen concentration.

Overall this result demonstrates that using the proposed approach can provide non-invasive and rapid in-situ measurements to quantify transport of oxygen across microencapsulation barriers.

Aim 2: Compare the oxygen diffusion barrier properties of microemulsion and microparticles:

In order to compare the results across different formulations and temperature conditions, the fluorescence data as shown in Figure 2(a) and 2(b) was normalized using ratio of fluorescence intensities as outlined in material and methods section. For quantitative comparison the normalized data was fitted using an exponential decay function. For WPI, the data shows a bi-exponential decay as described in material and methods sections. Similar trends have been observed in diffusion of oxygen in polymer matrices. Table 2 shows the values of the coefficients and the rate constants for the WPI microemulsions. For statistical comparisons only k_1 values will be used as they have much greater impact on the rate than k_2.

Figure 3 compares the ratiometric fluorescence decay for WPI and cross-linked WPI emulsions at (a) 25 °C and (b) 40 °C respectively. Comparison of rate constants (k1) for WPI and cross-linked WPI emulsion indicate that cross-linking of WPI microemulsion decreased the rate of fluorescence decay at both 25^{o}C and 40 °C ($p<0.05$).Similarly, the increase in temperature decreased the decay rates in both WPI and cross-linked WPI emulsion ($p<0.05$). Thus, both the factors, increase in temperature and cross-linking decreased the rate of oxygen transport rate across the barrier. Relative increase in the barrier properties of cross-linked WPI microemulsions as compared to non-crosslinked WPI emulsion can be attributed to a decrease in mobility of individual protein molecules as a result of chemical cross-linking. Thus, these results show that chemical cross-linking and temperature change (based on selected conditions in this study) can result in marginal changes in barrier properties of WPI microemulsion coatings. These changes although statistically significant may not make a significant impact on the oxidative stability of encapsulated products. . However, it is important to note that the proposed method is sensitive to detect small differences in oxygen transport in different formulation methods.

In order to compare the barrier properties of WPI microemulsions with different material formulation, SDS microemulsion was selected. SDS stabilized oil in water emulsions is a commonly used and studied system. In addition, the ability to modify

surface coating on SDS microemulsions using a layer-by-layer approach provides a model system to evaluate changes in the barrier properties with surface modifications. Figure 4 compares the normalized fluorescence decay in SDS and SDS-chitosan emulsions at (a) 25 °C and (b) 40 °C. Temperature showed no significant effect on the decay rate in either SDS emulsion or SDS-chitosan emulsion ($p>0.05$). Addition of chitosan layer over SDS emulsion significantly reduced the decay rate constant ($p<0.05$) at both, 25 and 40 °C. This suggests that layer by layer can marginally improve the oxygen transport rates. However, this improvement may not have any practical impact on the oxidative stability of the encapsulated compound.

Next, the primary emulsions (WPI and SDS emulsions) and modified emulsions (cross-linked WPI and SDS-chitosan emulsions) were compared in figure 5. It clearly demonstrates that WPI emulsion and cross-linked WPI emulsion have lower fluorescence decay rates than SDS emulsion and SDS-chitosan emulsion respectively. This suggests that, WPI offers a better barrier property against the oxygen transport than SDS emulsion. This difference in barrier properties of SDS and WPI can be possibly due to the fact that WPI (approximately 12-18000 Da) has a higher molecular size than SDS (288 Da), which may form a more homogenous and thicker coat around a droplet than SDS. Large molecular weight of WPI as compared to SDS may also reduce the mobility of WPI molecules at oil-water interface as compared to SDS. Despite differences in rate of oxygen transport with various formulations, the selected microemulsion compositions provided limited barrier to oxygen transport indicating high permeability of oxygen molecules across emulsified polymer barriers.

Summary: Fluorescence based method for measuring the diffusion of oxygen was developed and tested to study oxygen diffusion in WPI, cross-linked WPI, SDS and SDS-chitosan emulsions. The results showed that the method can be effectively used for quantitative in-situ measurements of rate of oxygen transport across microencapsulation barriers. The results also demonstrate that the proposed approach has high sensitivity to detect changes in structure (crosslinking, layer-by-layer assembly, temperature) of microencapsulation barriers. Measurement of oxygen transport demonstrated that WPI microemulsion has better barrier properties as

compared to SDS microemulsions. Overall, results of this study show that the selected composition of microemulsions provides a limited barrier to transport of oxygen. This highlights the need to develop better formulations to improve barrier properties and stability of encapsulated products. It is expected that the proposed approach can provide a rapid screening method to identify both optimal material and formulation methods to improve oxidative stability of encapsulated products.

Ongoing Work and Next steps: The next step in the proposed research is to compare microemulsions with solid lipid nanoparticles and coacervates in solution and in dry powder form. Ongoing work is focused on formulation and evaluation of these encapsulation methods. In addition, a proposal is submitted to CAAPS to extend the proposed technology to measure transport of free radicals and to relate transport of oxygen and free radicals with oxidation of encapsulated bioactive products.

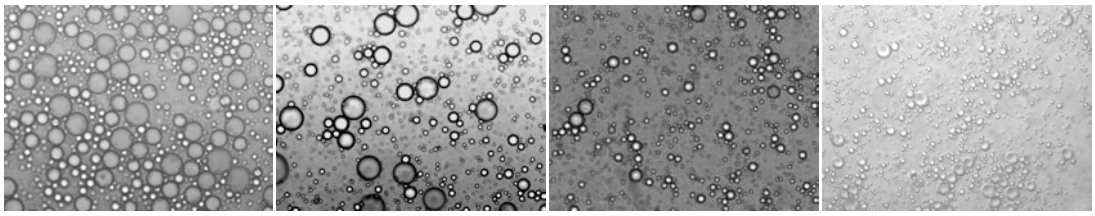
LIST OF FIGURES

TABLE 1

Emulsion	Average particle size (μm)	% population ≤ 50 μm
WPI emulsion	22.5	91
Cross-linked WPI emulsion	31.5	85
SDS emulsion	12.4	100
SDS-chitosan emulsion	114.2	49

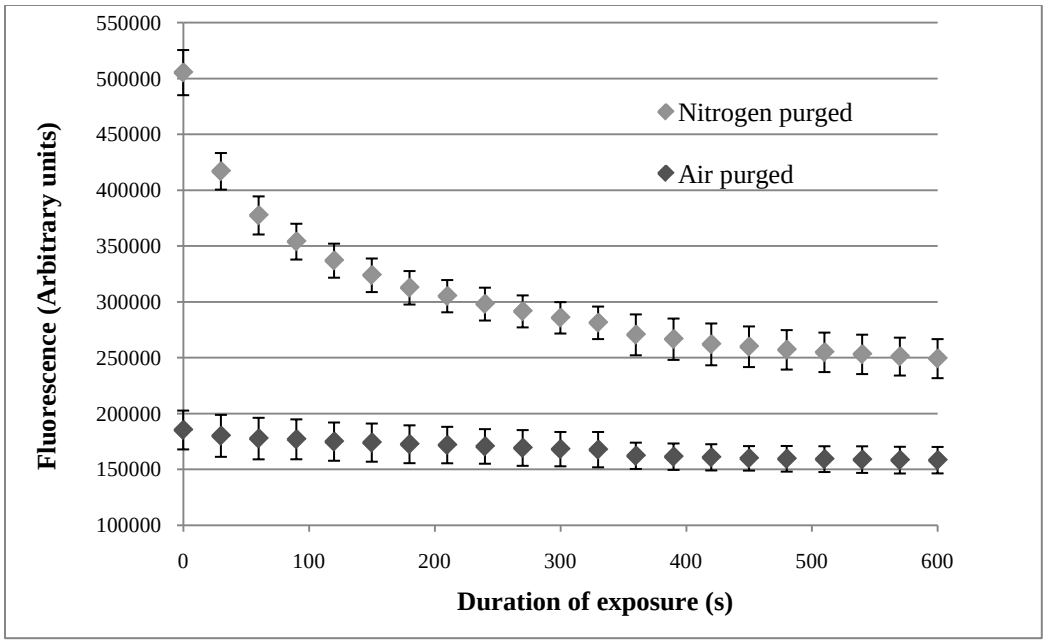
TABLE 2

Emulsion	a	k_1 ($\times 10^{-3}$)(s^{-1})	c	k_2 ($\times 10^{-3}$)(s^{-1})
WPI at 25 °C	47.91	20.39	51.34	1.07
WPI at 40 °C	36.25	13.3	62.78	0.59
Cross-linked WPI at 25 °C	33.55	14.85	65	0.8
Cross-linked WPI at 40 °C	29.96	8.59	68.77	0.82
SDS at 25 °C	99.09	7.6	-	-
SDS at 40 °C	98.13	6.8	-	-
SDS-chitosan at 25 °C	98.1	3.6	-	-
SDS-chitosan at 40 °C	99.4	4.6	-	-



(a) (b) (c) (d)

Figure 1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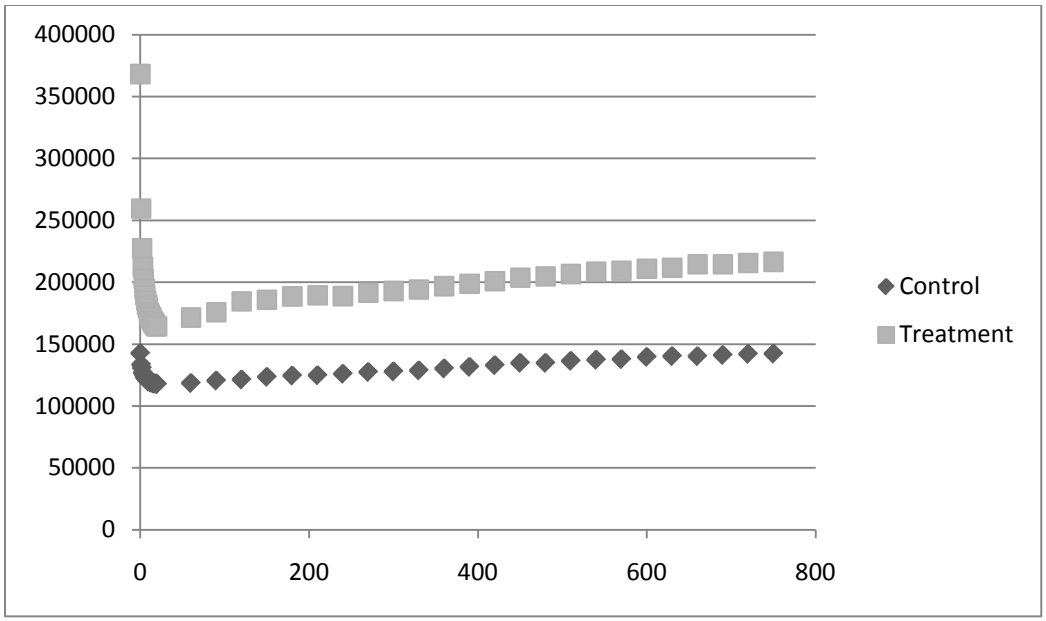
500000
450000
400000
350000
300000
250000
200000
150000
100000
Fluorescence (Arbitrary units)
Nitrogen purged
Air purged
0
100
200
300
400
500
600
Duration of exposure (s)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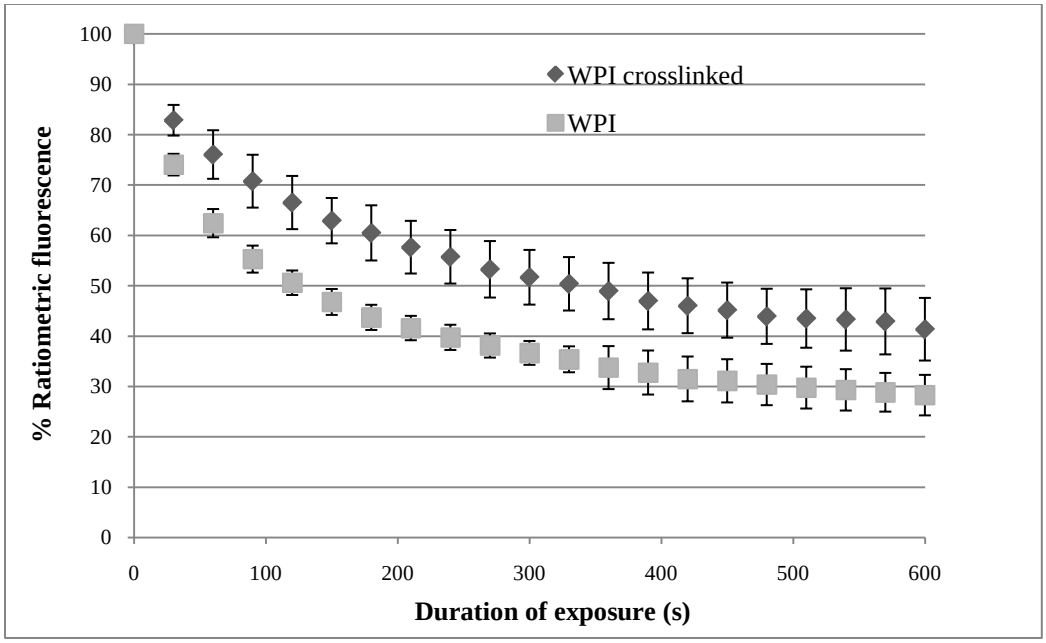
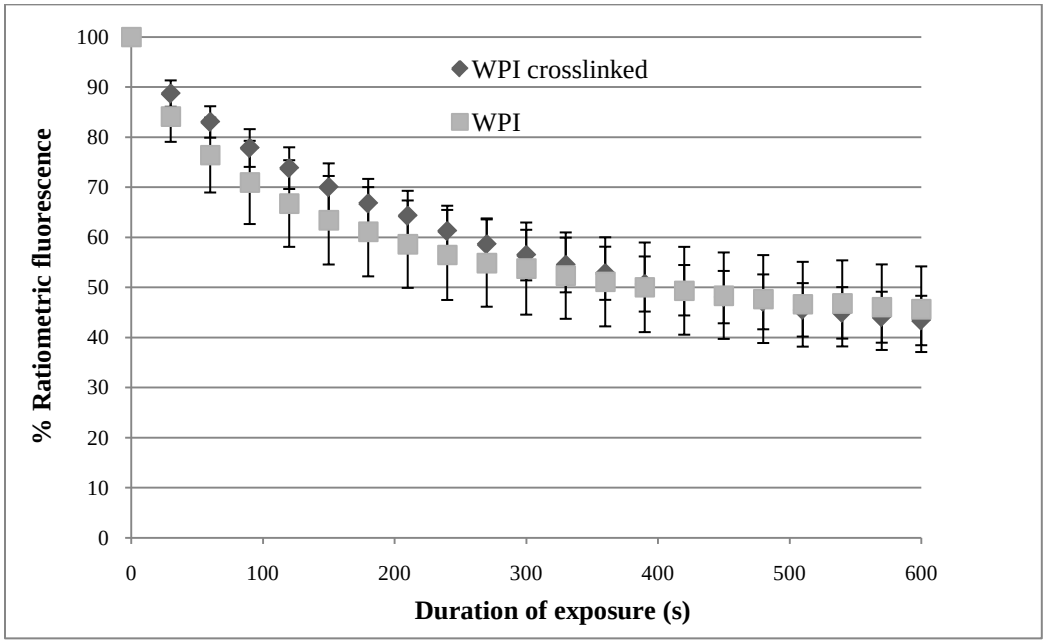
200
400
600
800
Control
Treatment

Figure 2


100
90
80
70
60
50
40
30
20
10
0
% Ratiometric fluorescence
WPI crosslinked
WPI
0
100
200
300
400
500
600
Duration of exposure (s)

(a)


100
90
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70
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40
30
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10
0
% Ratiometric fluorescence
WPI crosslinked
WPI
0
100
200
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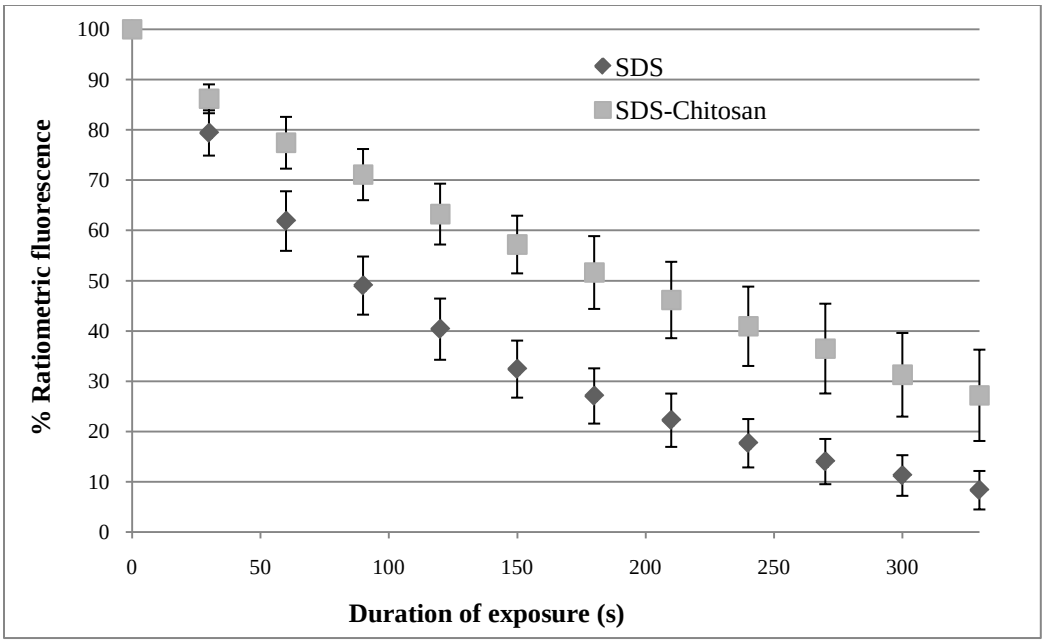
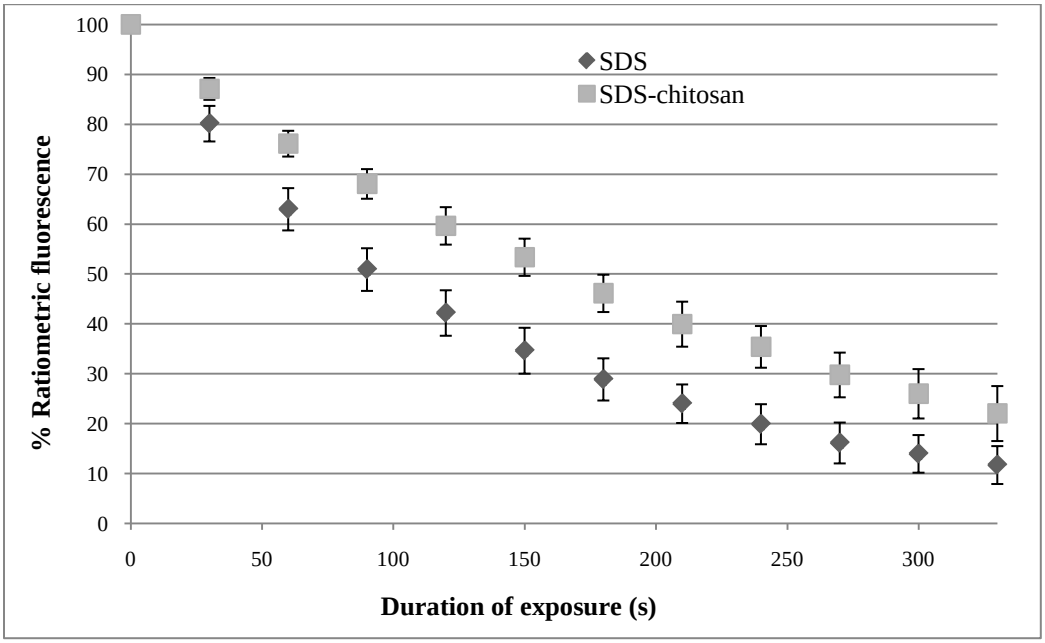
400
500
600
Duration of exposure (s)

(b)

Figure 3


100
90
80
70
60
50
40
30
20
10
0
% Ratiometric fluorescence
SDS
SDS-Chitosan
0
50
100
150
200
250
300
Duration of exposure (s)

(a)


100
90
80
70
60
50
40
30
20
10
0
% Ratiometric fluorescence
SDS
SDS-chitosan
0
50
100
150
200
250
300
Duration of exposure (s)

(b)

Figure 6